Developing New Methods for Rapid Serial Crystallography

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Introduction

The availability of very high intensity synchrotron sources is increasingly motivating a change of the traditional single-specimen paradigm for macromolecular crystallography. At existing third generation synchrotrons, data collection speed is limited by mechanical constraints and not by photon flux because single crystal specimens must be precisely rotated. Users of brilliant X-ray sources such as the Advanced Photon Source attenuate their beams by up to 99% so that mechanical and electronic components can keep up. This technology will employ the full intensity of NSLS-II to discover novel structures using slurries of micro crystals that are currently not suitable for conventional single-crystal experiments. We aim to demonstrate that crystal structures can be readily solved from many randomly oriented micro crystals.

Data Table 1

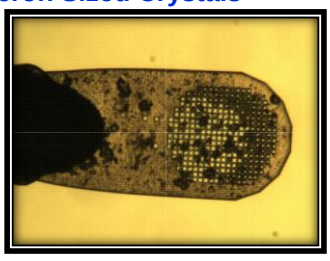
Number of Crystals	127 (25µm)
X-ray source	NSLS (Beamline X12B)
Wavelength	1.27 Å
Space group	R3
Unit cell parameter	a=b=81.7Å, c=33.8Å
Resolution	1.7Å
R-merge	15.0%
Percentage of Completeness	85.8%
Redundancy	9.7

Raster Scan Method

In this experiment we are using insulin micro crystals (~25µm, space group *R3*) which were grown overnight from an established crystallization protocol that was modified to produce micro crystals. The crystals were cryo mounted (100K) using MiTeGen micro meshes and there were about 50 crystals on each mesh. Data were collected using the CBASS program and processed with HKL2000. Custom software was developed to rapidly scan the mesh though the X-ray beam, and to help examine the resulting data. The 400 micron square area of the MiTeGen mesh was divided into equal fields with dimensions similar to the majority of the crystals (20 microns), and each field was exposed for 3 rotations of 1 degree, for 90 seconds each. Useful data were partitioned from poor or empty fields by inspection, aided by custom software.

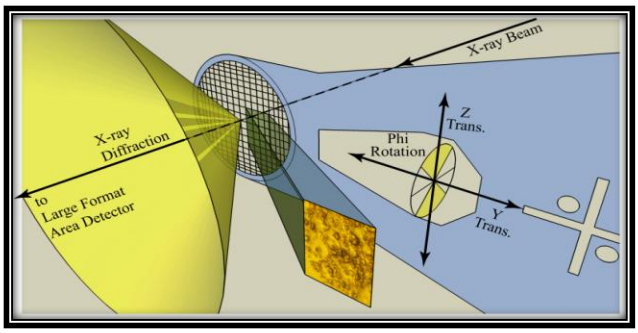
X-ray Diffraction from Micron Sized Crystals

a. Insulin micro crystals grown using the batch method (scale bar = 25µm).



b. MiTeGen Micromesh(25µm holes) holding crystals.

Raster Scan Serial Crystallography

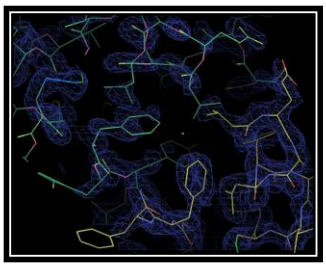


a. The X-ray Beam passing through the MiTeGen Micromesh (25µm). The mesh was scanned across the beam, so that each 20x20 micron field was exposed for 3 images exposed for 90 second with 1 degree rotations.

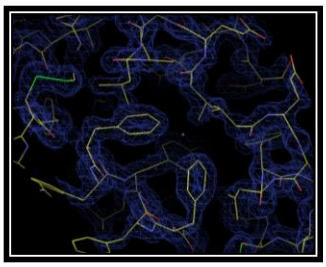
Result

Overall, 800 data sets (2400 images) were examined, with about 1/3 of these containing usable diffraction data. A single working set was generated by combining data from 127 different micro crystals on two 400 micron meshes. Various attempts to obtain a SAD solution failed due to the limited completeness, but the micro crystal data readily yielded a highly interpretable map when phased by **molecular replacement.**

Insulin structure



a. Initial molecular replacement map from MolRep (CC = 74.62%)



b. Refined insulin structure from 127 micro crystals (25µm) solve by molecular replacement (R =22.34, Rfree =27.68)

Data Table 2

Insulin protein
Crystallization Shower → Serial Crystallography
Data Collection → Custom software in CBASS
Data processing→ HKL2000
Structure solution → CCP4/Molrep / Coot
Phase Refinement→ RefMac

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